83-3 - Rabbit - Teratogenicity

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DATA EVALUATION REPORT

Study Type: Rabbit Teratology

TOX. CHEM. No.: 2980

Accession No.:

MRID No.: 400288-16

Test Material: CGA 154281 Technical (FL 860318; 93.9% Purity)

Study Number(s): 483-246

Sponsor: CIBA-GEIGY Corp.

Test Facility: Hazleton Laboratories America, Inc.

Title of Report: Rabbit Teratology Study with CGA 154281

Author(s): S.L. Morseth, C.M. Slabik, L.M. Lichtenberger, K.J. Vargas, S.A. Lewis, A.K. Thakur, and P.L. Burlew

Report Issued: October 10, 1986

Conclusions:

Maternal Toxicity NOEL = To be determined

Developmental Toxicity NOEL = To be determined

Dose levels tested: 0, 0.5, 2.5, 12.5, and 62.5 mg/kg/day.

Deficiencies in reporting of the skeletal malformations, which are identified in the detailed review, should be clarified and resolved.

Classification of Data: Supplementary

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400 28815

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Title of Report: Rabbit Teratology Study with CGA 154281 Technical

Procedure:

The method used to determine the embryo/fetal toxicity and teratogenic potential of CGA 154281 Technical in pregnant rabbits is outlined below:

(1) Sexually mature, virgin HRA:SPF female rabbits were artificially inseminated. Each rabbit was inseminated a second time approximately 3-4 hours after the first insemination in order to ensure a good pregnancy rate. The day of insemination was designated as day 0 of gestation.

(2) Five groups of pregnant rabbits, 15 per group, were orally intubated with CGA 154281 Technical, suspended in water with 0.5% carboxymethyl cellulose and 0.1% Tween 80, at 0.5, 2.5, 12.5, and 62.5 mg/kg/day for 13 consecutive days (initiated on day 7 and continuing through day 19 of gestation). The control animals received carboxymethyl cellulose (0.5%) and Tween 80 (0.1%) concurrently with the test compound.

(3) Statistical Analysis: The following procedures were utilized in analyzing the numerical data: (a) When variances of untransformed data were heterogenous, analyses were performed on transformed data to achieve variance homogeneity; (b) When the services of transformation were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data; (c) The criterion for significance of groups comparison was routinely at the 5% two-tailed probability level, however, in the case of significant trend, the criterion was defined at the 5% one-tailed probability level.

Methods and Results:

(1) Individual maternal observations were recorded daily for mortality and moribundity. Clinical observations on all females were also conducted daily throughout the study.

Results: There were no significant differences in clinical signs found between the treated animals and control animals. However, the incidences of animals with anorexia were slightly greater in the 62.5 mg/kg/day dose group when compared to that of the control group. All rabbits survived during the period of this study.

(2) Maternal body weights were measured on gestation days 0, 7, 9, 11, 15, 20, 24, and 29.

Results: Mean maternal body weights and body weight changes were similar among all groups at each interval measured.

(3) Food consumption was recorded on *gestation* days 0, 7, 9, 11, 15, 20, 24, and 29.

Results: Summary of Maternal Food Consumption (g) - Mean Values

Dose Level	0	0.5	2.5	12.5	62.5 (mg/kg/day)
Days 0-7	1192	1220	1112	1274	1187
Days 7-9	360	359	329	365	297
Days 9-11	315	324	308	325	267
Days 11-15	561	506	556	585	373*
Days 15-20	777	673	758	731	604
Days 20-24	608	551	543	539	591
Days 24-29	515	482	431	453	634

* Significantly different from control value, $P<0.05$.

Findings: Significantly decreased mean food consumption value at the 62.5 mg/kg/day dose group was noted as compared to that of the control group during the day 11-15 interval. All other mean food consumption values were similar to the corresponding control values during the period of gestation (days 7 to 29).

(4) Observations at terminal *necropsy:* Dark red stomach lining, mottled and/or pale liver and hyperdistention of the urinary bladder were found in both the control and treated animal groups. These findings are considered to be incidental and not compound-related.

(5) Uterine Weights: There were no significant differences in *mean* uterine weights found between the control and treated animal groups.

(6) Maternal Survival and Pregnancy Status:

Results:

Dose Level	0	0.5	2.5	12.5	62.5 (mg/kg/day)
No. of females inseminated	15	15	15	15	15
No. of females pregnant	14	14	14	14	14
(%)	(93)	(93)	(93)	(93)	(93)
No. of females aborting	0	1	0	0	1
(%)	(0)	(7.1)	(0)	(0)	(7.1)

Findings: There were no treatment-induced effects in the maternal survival and pregnancy status observed among the dams in the treated groups.

(7) Mean Fetal Data:

Results:

Dose Level	0	0.5	2.5	12.5	62.5 (mg/kg/day)
No. of litters examined	14	13	14	14	13
Mean No. of:					
Corpora lutea	11.1	11.2	10.7	11.6	13.2
Implantations	7.4	7.6	6.1	7.9	8.8
Live fetuses	6.7	6.6	5.9	7.1	8.3
Dead fetuses	0.1	0	0	0	0
Early resorption	0.6	0.8	0.3	0.8	0.5
Late resorption	0	0.2	0	0	0.1
Male fetuses	2.9	3.6	2.6	3.4	4.5
Mean live fetal weight (g)	46.4	47.4	48.0	46.4	43.3
Mean male weight	45.7	47.3	46.1	46.3	44.4
Mean female weight	46.0	46.5	47.0	46.3	40.9
Covariate adjusted:					
Mean live fetal weight	46.2	46.8	45.8	46.7	46.1
Mean male weight	45.5	46.7	44.4	46.7	46.5
Mean female weight	45.4	46.3	44.6	46.2	44.2

Findings: No statistically significant differences in the mean number of viable fetuses, early and late absorptions, implantation sites, and corpora lutea were observed between the control and treated animal groups. The fetal sex distribution was unaffected by the treatment of CGA 154281 Technical in the pregnant rabbits. Fetal body weights appeared slightly lower for the high dose animal group (62.5 mg/kg/day) when compared to the control value. However, this difference is not statistically significant when the litter size is used as a covariate in the analysis.

(8) Fetal Morphology:

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Dose (mg/kg)	Fetuses					Litters				
	0	0.5	2.5	12.5	62.5	0	0.5	2.5	12.5	62.5
Variations:										
(A) No. Examined Externally	95	86	82	99	108	14	13	14	14	13
Small Domed Head	0	1	0	0	0	0	1	0	0	0
(B) No. Examined Viscerally	95	86	82	99	108	14	13	14	14	13
Intermediate Lobe Missing/Small	5	7	5	6	5	4	4	3	5	3
(C) No. Examined Skeletally	95	86	82	99	108	14	13	14	14	13
Bipartite Hyoid Body	3	2	0	4	2	2	1	0	3	4
Unossified Hyoid Body	0	0	1	0	1	0	0	1	0	1
24 Presacral Vertebrae	0	1	1	1	1	0	1	1	1	1
26 Presacral Vertebrae	14	16	5	28	7	7	5	4	10	4
Bipartite Vertebral Centrum	0	0	1	0	1	0	0	1	0	1
5th Sternebrae Unossified	1	10	0	3	8	1	7	0	2	5
11th Full Pairs of Ribs	0	0	0	0	2	0	0	0	0	2
13th Full Pairs of Ribs	26	28	12	35	19	10	8	5	12	9
Malformations:										
(A) No.Examined Externally	95	86	82	99	108	14	13	14	14	13
Gastroschisis	0	0	0	0	1	0	0	0	0	1
Shortened Tail	0	0	0	0	1	0	0	0	0	1
(B) No Examined Viscerally	95	86	82	99	108	14	13	14	14	13
Heart and/or Great Vessel Anomaly	2	1	0	0	0	2	1	0	0	0
(C) No. Examined Skeletally	95	86	82	99	108	14	13	14	14	13
Vertebral Anomaly with or without Associated Rib Anomaly	1	2	2	3	7	1	2	2	3	7
Short Tail Noted	0	0	0	0	1	0	0	0	0	1
Misaligned and Fused Caudal Vertebrae	0	0	0	0	2	0	0	0	0	1

Summary of Findings:

1. External Examinations - One fetus with a small domed head was observed in the 0.5 mg/kg/day dose group. Another fetus was malformed with a shortened tail and gastroschisis in the 62.5 mg/kg/day dose group.

2. *Visceral Observation - Missing or small intermediate lobes were observed in all treated and control fetuses. There were no differences in the visceral variations and malformations found between the control and treated fetuses.*

3. Skeletal Examination - The skeletal variations more commonly observed were 26 presacral vertebrae, unossified 5th sternebrae and 13th full pair of ribs among all fetuses of the treated and control groups. These incidences with respect to both fetuses and litters did not reflect a dose-related response. However, there were apparently dose-related increases in vertebral anomaly with or without associated rib anomaly observed in the treated groups receiving 0.5, 2.5, 12.5 and 62.5 mg/kg/day of CGA 154281 Technical. The frequency of this type of skeletal malformation was highest at the highest dose level (62.5 mg/kg/day). Other skeletal malformations included misaligned and/or fused caudal vertebrae and a minor malformation involving vertebrae near the base of the tail in the two fetuses of 62.5 mg/kg/day dose group (Litter No. E40963).

Conclusion:

1. The parameters which were unaffected by the treatment of CGA 154281 Technical in the pregnant rabbits included the clinical observation, maternal body weight change, uterine weight, necropsy findings, death of fetuses, abortion, pregnancy status, resorption, implantations, and fetal body weight.

2. Although the food consumption values were significantly decreased in the 62.5 mg/kg/day dose group during the days 11-15 interval, no other difference for the food consumption values was found between the control group and the highest dose group during the remaining gestation period (i.e., days 15-20; days 20-24; days 24-29 intervals). Therefore, it may be concluded that the maternal toxicity was not induced by the test compound at the dose levels tested (0.5 through 62.5 mg/kg/day) in this study.

3. Developmental toxicity appears to be demonstrated by the increased incidences of vertebral anomaly with or without associated rib anomaly in a dose-related manner from the dose groups receiving 0.5, 2.5, 12.5, and 62.5 mg/kg/day of CGA 154281 Technical. Other skeletal malformations including misaligned and fused caudal vertebrae and a minor malformation involving vertebrae near the base of the tail in the two fetuses of highest dose group. However, the evaluation of skeletal malformation cannot be accomplished due to the following reporting deficiencies:

   A. Pg. 9 of the report which is pg. 17 of the volume states: " A second skeletal evaluation was performed on each fetus from the control and high dose groups in order to verify the incidences of vertebral malformations ". But, the detailed data for the second evaluation cannot be found in the report.

B. The historical control findings (Appendix 13) identified as vertebral arches and/or central malaligned or fused do not appear to be equivalent to thoracic vertebral malformations found in this study. Further clarification with respect to the incidences of specific skeletal malformations (i.e., vertebral anomaly with or without associated rib anomaly) found in this study is needed for proper evaluation. These historical control data must be provided from the same strain of rabbits and suppliers., during the same time period (+ or - 2 years), and should include information concerning gavage vehicles, etc.

C. Since the increased frequency of thoracic vertebral malformations was detected in a dose-related manner in this study, correct grouping of vertebral or axial skeleton findings must be provided for the proper evaluation of developmental toxicity of the test compound.

Classification of Data: Supplementary

Maternal Toxicity NOEL = To be determined

Developmental Toxicity NOEL = To be determined

The study cannot be upgraded until all the reporting deficiencies cited in our conclusion #3 are clarified and resolved.